Molecular Ecology (2002) **11**, 2307–2316

Nestmate relatedness and population genetic structure of the Australian social crab spider *Diaea ergandros* (Araneae: Thomisidae)

THEODORE A. EVANS* and MICHAEL A. D. GOODISMAN†
*Department of Zoology, University of Melbourne, Parkville, VIC 3052, Australia, †Department of Zoology and Tropical Ecology, James Cook University, Townsville, QLD 4811, Australia

Abstract

We characterized the population genetic structure of the Australian social spider *Diaea ergandros* using polymorphic allozyme markers. Our main objectives were to understand the social organization of *D. ergandros* and discern patterns of gene flow across distantly separated geographical areas. Spiders were sampled from nests located within 100 m wide locales, which were distributed within larger 50 km wide regions. Our results indicated that nestmates could have been produced by a single mother and father in 88.9% of *D. ergandros* nests. The remainder of nests contained spiders that were probably produced by polyandrous females or were immigrants from foreign nests. Nestmate relatedness was relatively high ($r = 0.44$) and did not differ significantly between the sexes or among juvenile, subadult and adult life stages. We also discovered that *D. ergandros* populations were highly structured, with significant differentiation detected among locales ($F_{LR} = 0.23$) and regions ($F_{RT} = 0.081$). Spiders within locales were also substantially inbred ($F_{IL} = 0.15$). Overall, our data show that significant population subdivision exists in *D. ergandros* populations, and we suggest that the poor dispersal ability of *Diaea* spiders can account for the observed genetic structure.

Keywords: gene flow, kin selection, mating system, migration, polymorphic allozyme, sociality

Received 20 March 2002; revision received 7 August 2002; accepted 7 august 2002

Introduction

Sociality in many arthropods is believed to have evolved under kin selection, whereby social individuals increased their inclusive fitness by helping relatives (Hamilton 1964; Crozier & Pamilo 1996; Choe & Crespi 1997). Under this scenario, the evolution of social behaviour relied critically on kin living in proximity, so that related individuals would be in a position to cooperate. Previous studies have tested this assertion by examining genetic structure in social insect or arachnid taxa to determine if relatives are indeed clumped in space. Most of these investigations restricted analysis to the highly social Hymenoptera (Crozier & Pamilo 1996; Bourke & Franks 1995) and discovered that related individuals do interact within colonies. However, the focus on only this single order and on mainly eusocial taxa leaves a serious gap in our knowledge of genetic structure in other social arthropods. The investigation of population structure in other groups would probably contribute substantially to our understanding of the conditions under which sociality may have evolved.

Knowledge of genetic structure in social spiders would be particularly insightful. The vast majority of the more than 30 000 described species of spiders are solitary and aggressive, thus the presence of a *c*. 90 species of spiders in 12 families that display some form of grouping behaviour is especially intriguing (d'Andrea 1987; Avilés 1997; Uetz & Hieber 1997). The majority of these social spiders live in communal webs and may cooperate in nest construction and prey capture. Social behaviour in spiders has been classified according to whether spiders maintain individual territories and whether the duration of group living persists for the entire life cycle (d'Andrea 1987; Avilés 1997; both after Kullman 1968). Those considered as having the

Correspondence: Michael A. D. Goodisman, Department of Biochemistry and Molecular Biophysics, 513 Biological Sciences West, PO Box 210088, University of Arizona, Tucson, AZ 85721–0088, USA. Fax: 520-621-9288; E-mail: goodisma@email.arizona.edu
Current address: CSIRO Entomology, Canberra, ACT 2601, Australia.

most derived type of group-living display 'nonterritorial and permanently social' (d'Andrea 1987) behaviour; these spiders do not maintain individual territories on the colonial web and have overlapping generations in the same colony (i.e. mature females may remain within their natal colonies to reproduce). Inbreeding over many generations is believed to maintain relatedness among spiders cohabiting within these webs and is thought to contribute to the evolution and maintenance of sociality (Smith & Hagen 1996; Avilés 1997). Although this claim is feasible, there are no comparisons with social spiders in categories considered to have less derived forms of sociality, and so the validity of this assertion remains relatively untested.

Spiders in the genus *Diaea* provide opportunities to investigate the genetic structure of species displaying less complex social systems. *Diaea* spiders differ from other well-studied 'nonterritorial, permanently social' spiders in that they always migrate from their natal nests to breed, which places them in the 'nonterritorial, periodically social' category (Avilés 1997). Moreover, the social *Diaea* are worthy of study because they lack the snare web considered by some to be essential to sociality (Kullman 1968, 1972; Shear 1970), and because they are 'similar to the highly inbred cooperatively spiders and appear to be at the transition point between periodic- and permanently sociality' (Avilés 1997).

The spider *Diaea ergandros* inhabits the foliage of *Eucalyptus* species in the forests on the southeast coast of Australia (Main 1988; Evans 1995, 1997). *D. ergandros* mature gravid females disperse from their natal nest alone to found their own nests. They construct a leaf nest at the growing tip of a branchlet, beginning with a single curled leaf that serves as a brood chamber and then wrapping other leaves around it. The female lays a single egg sac and devotes the remainder of her life to caring for these offspring, including the extreme sacrificial act of matriphagy (Evans *et al*. 1995; Evans 1998a,b). The leaf nest is essential for survival, as it serves as a protective retreat from predators and as a foraging area, because crab spiders are ambush predators and do not build webs (Evans 1998a). The leaf nest lasts only a single year and will not be reused after the mother has died and the offspring have dispersed (Evans 1998a, 1999).

Although the group inside the nest originates as a single family, unrelated immigrants may be accepted into the nest (Main 1988; Evans 1999). *D. ergandros* spiderlings do not disperse when very young, because they receive extended maternal care. Therefore they cannot balloon, but instead walk along branches; late instar juvenile and subadult spiders have been observed moving between nests in the field. Spiders from different nests do not show more obvious aggression than those taken from the same nest in the laboratory and, as seen for other social spiders, all individuals contribute to nest construction (Evans 2000). The end result of this presumed migratory behaviour and lack of aggression is that nestmate relatedness may degrade as the colony ages.

The aim of this study was to understand the organization of genetic variation in *D. ergandros* populations. Specifically, we sought to determine the relatedness of spiders within nests and the differentiation of spiders found in distinct local areas and broader geographical regions. The complex life history of *D. ergandros* suggests that populations may be subdivided and structured around kin groups, although this may change with the age of the colony. Indeed, group relatedness should be high, at least in young groups, because spiders inhabiting nests should be the offspring of one female (Evans 1998a). Moreover, we expect that spiders in local areas might be quite closely related, and that gene flow to other more distance areas will be low because of the relatively poor dispersal abilities of the spiders.

Materials and methods

Sampling scheme

A total of 1122 *Diaea ergandros* spiders were collected from June to October (winter to spring) 1993, throughout the spider's range in southeastern Australia (Fig. 1). The sampling programme was hierarchical. Up to eight

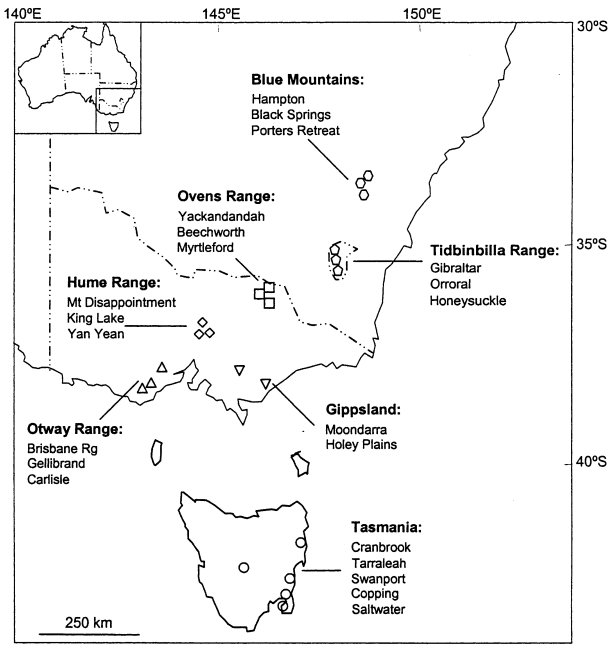

Fig. 1 Southeastern Australia showing locations of regions and locales from which *D. ergandros* spiders were sampled. Locales from the same region are signified by distinct symbols. The Blue Mountains are represented by hexagons, the Tidbinbilla Range by pentagons, the Ovens Range by squares, the Hume Range by diamonds, the Otway Range by triangles, Gippsland by upside-down triangles and Tasmania by circles. Locales are listed under regions in order of decreasing latitude. State borders are indicated by dotted lines.

Table 1 Locations (latitude °S, longitude °E) of locales from which *D. ergandros* spiders were collected. The number of nests, number of spiders, and range in number of spiders sampled per nest within each locale are also given

Region, locale	Location	Nests	Spiders	Range
Blue Mountains				
Hampton	33°40′, 150°03′10	10	71	1–8
Black Springs	33°52′, 149°44′	10	52	1–8
Porters Retreat	34°03′, 149°50′	10	81	5–8
Tidbinbilla Range				
Gibraltar	35°32′, 148°53′	11	59	1–8
Orroral River	35°35′, 149°00′	7	45	3–8
Honeysuckle Creek	36°20′, 146°52′	9	55	2–8
Ovens Range				
Yackandandah	35°39′, 148°57′	10	54	1–8
Beechworth	36°22′, 146°36′	1	8	NA
Myrtleford	36°35′, 146°40′	7	52	3–8
Hume Range				
Mt Disappointment	37°20′, 145°10′	10	51	3–8
Kinglake	37°32′, 145°21′	9	54	1–8
Yan Yean	37°33′, 145°09′	12	87	4–8
Otway Range				
Brisbane Range	37°52′, 144°14′	12	85	4–8
Gellibrand	38°32′, 143°32′	7	49	1–8
Carlisle	38°35′, 143°22′	6	38	2–8
Gippsland				
Moondarra	38°07′, 146°17′	5	34	5–8
Holey Plains	38°12′, 146°50′	7	47	4–8
Tasmania				
Tarraleah	42°19′, 146°26′	7	33	1–8
Saltwater	43°01′, 147°42′	7	55	7–8
Copping	42°49′, 147°46′	7	37	1–8
Swanport	42°20′, 147°57′	3	6	1–8
Cranbrook	42°01′, 148°05′	10	69	6–8

NA, not applicable.

individuals (*c*. 25% of the nest occupants) were sampled from 178 nests in 22 locales within seven larger regions (Table 1). A maximum of 10 nests was collected in each locale (~100 m × 100 m). Up to three locales separated by no more than 20 km were sampled within a region, and the distance between any two regions always exceeded 100 km.

In most nests, the life stage of the spiders present could be determined. The majority of spiders were consequently categorized as juveniles (instars 1–3), subadults (male instar 4 and female instar 4 and 5), or adults (male instar 5 and female instar 6). In some instances, a single adult female was present in conjunction with several younger spiders. This adult female was taken to be the putative founding mother of the nest.

Electrophoretic methods

Individual whole spiders were assayed for allozymic variation by horizontal starch gel electrophoresis following Richardson *et al*. (1986). Chilled spiders were ground in three drops of buffer solution; filter paper inserts (4 × 6 mm) were used to transfer the extract to the starch gels. Gels (150 × 150 × 6 mm) were made from potato starch (Starch Art) and could take 24 inserts. Each gel had 16 unique and eight replicate inserts. Selected enzymatic extracts from different regions and from all grinding plates were re-run for confirmation of loci.

Twenty-seven enzyme systems were screened on up to four buffers to determine the best resolution. The following enzymes did not give scorable results: adenylate kinase, creatine kinase, glutamate-oxaloacetate-transminase, leucine aminopeptidase, L-leucyl-proline and malate dehydrogenase (DH). The following enzymes were scored as monomorphic: alcohol DH, arginine phosphokinase, catalase, glutamate DH, α-glycerophosphate DH, guanine deaminase, hexokinase, L-leucyl-glycylglycine, L-leucyl-L-tryosine, L-valyl-leucine, malic enzyme, sorbitol DH and superoxide dismutase. The following enzymes were polymorphic, but were not consistently clear enough to score

reliably: esterase, glucose-6-phosphate DH, mannose-6-phosphate isomerase and phosphoglucomutase. Finally, four putative loci were polymorphic and clear enough to score reliably in *D. ergandros*; these were isocitrate DH (Idh), lactate DH (Ldh), 6-phosphogluconate DH (6Pgd) and phosphoglucose isomerase (Pgi).

Genetic analyses

Allele frequencies within locales were estimated using the program RELATEDNESS 4.2 (Queller & Goodnight 1989), with nests weighted equally. The mean expected heterozygosity of each locus was obtained by taking the mean of the expected heterozygosities within each locale and provided an estimate of the variability of the markers.

The relatedness of spiders within nests was also calculated with RELATEDNESS 4.2. Nests were again weighted equally and the 'deme' function was used to control for allele frequency differences among locales. Two locales (ACT-Gibralter and ACT-Honeysuckle Creek) were not scored for IDH and were therefore not used in these analyses. Standard errors for the relatedness estimates were obtained by jackknifing over nests. Relatedness estimates were judged to differ significantly from 0.0 or 0.5 if their 95% confidence intervals ($r \pm 1.96$ SE) failed to overlap these values. Independent estimates of nestmate relatedness were obtained for spiders of both sexes, as well as for each of the three age classes (juveniles, subadults and adults). A Mann–Whitney *U* or Kruskal–Wallis test was used to test for differences in the nest-level relatedness estimates between the two sexes and among the three age classes, respectively.

The genotypes of nestmates were examined directly to determine if the cohabiting spiders comprised simple families. Here, a simple family was defined as including only full siblings. If a putative mother was not present within the nest, then we tested if the genotypes of nestmates were consistent with having been produced by a single unsampled female and a single male. If the putative mother was present, then we tested to see if that female and a single male could have produced all other nestmate genotypes.

A reduced data set obtained by randomly selecting a single spider per nest was used in all subsequent population analyses to obtain a genotype distribution unbiased by nest-level structure. Probability or score tests (Rousset & Raymond 1995) as implemented by the program GENEPOP 3.1c (Raymond & Rousset 1995) were used to examine the significance of Hardy–Weinberg disequilibrium within locales. Both heterozygote deficit and excess were considered as alternate hypotheses.

To examine the higher-level structure of *D. ergandros*, Wright's hierarchical *F* statistics, F_{IL}, F_{LR}, F_{RT} and F_{IT}, were estimated by Weir's (1996) f, θ_S, θ_P and F, respectively, using the program Genetic Data Analysis (Lewis & Zaykin 2000). Under the sampling scheme used in this study, I = individual, L = locale, R = region and T = total population. Each *F*-statistic measured the decrease in heterozygosity at different levels of the population hierarchy due to true inbreeding (F_{IL}) or population structure (F_{LR} or F_{RT}) or both of these factors (F_{IT}). The 95% confidence intervals around the estimates were obtained by bootstrapping over loci 10 000 times and estimates were considered to be significantly different from zero if the 95% confidence intervals did not overlap zero.

The relationship between geographical distance and genetic similarity of locales was assessed to determine if *D. ergandros* displayed genetic isolation by distance. The program GENEPOP 3.1c was used to calculate genetic differentiation between all pairs of locales, as measured by the statistic F_{LT}. The relationship between the geographical distance and genetic similarity of locales was measured by Spearman's rank order correlation coefficient, r_S. The significance of the association was determined by a Mantel test, with 10 000 permutations, as conducted by GENEPOP 3.1c. The *P*-value obtained from this test gives the probability that a random correlation obtained from the permutated data set was larger than that observed.

Finally, the neighbour-joining algorithm (Saitou & Nei 1987), as implemented by the program PHYLIP 3.572c (Felsenstein 1989), was used to assess the genetic relationships among *D. ergandros* locales. An unrooted dendrogram was constructed based on Cavalli-Sforza's chord distances (Cavalli-Sforza & Edwards 1967) between locales for which all loci were scored. The data were bootstrapped over loci 1000 times to provide estimates of reliability for the nodes on the resulting consensus tree.

Results

Variability of markers

In *Diaea ergandros*, the loci *Idh*, *6Pgd*, *Pgi* and *Ldh* exhibited four, four, four and three alleles, respectively (Table 2), with associated average expected heterozygosities of 0.30, 0.47, 0.38 and 0.036. One or two alleles at each locus predominated through most of the population.

Relationships of nestmates

All relatedness estimates for *D. ergandros* nestmates reached relatively high levels and were significantly greater than zero (Table 3), indicating that nests usually contained kin. However, the values for all individuals, males, females, and subadults were significantly less than 0.5, the minimum value expected if nestmates comprised simple families. There are at least two nonexclusive explanations for this result. First, the original founding mother of the nest could have mated with multiple males.

Table 2 Allele frequencies at four polymorphic loci assayed in the spider *D. ergandros*

	Pgi				6Pgd				Idh				Ldh		
Region, localec	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c
Blue Mountains															
Hampton	0.013	0.192	0.796		0.196	0.294	0.498	0.013	0.325	0.587	0.087			1.000	
Black Springs		0.211	0.760	0.029	0.011	0.101	0.768	0.119	0.231	0.638	0.132			1.000	
Porters Retreat		0.232	0.712	0.056	0.011	0.55	0.383	0.056	0.226	0.599	0.175			1.000	
Tidbinbilla Range															
Gibralter		0.269	0.658	0.073	0.076	0.034	0.646	0.243					0.028	0.972	
Orroral Rv		0.199	0.741	0.060	0.036	0.842	0.098	0.024	0.360	0.399	0.241			1.000	
Honeysuckle		0.102	0.836	0.062		0.174	0.688	0.139	1.000				0.014	0.935	0.051
Ovens Range															
Yackandandah		0.363	0.637		0.014	0.511	0.454	0.021	1.000					1.000	
Beachworth		0.062	0.938			0.500	0.062	0.438	0.312	0.688				1.000	
Myrtleford		0.419	0.581		0.334	0.404	0.146	0.116	0.096	0.904				1.000	
Hume Range															
Kinglake		0.303	0.697		0.021		0.504	0.475	0.171	0.829				1.000	
Mt Disappointment	0.247	0.095	0.657			0.065	0.453	0.482	0.085	0.798	0.031	0.085		0.994	0.006
Yan Yean		0.281	0.701	0.018	0.057	0.442	0.501	0.106	0.778			0.116	0.021	0.979	
Otway Range															
Brisbane R	0.005	0.106	0.712	0.177	0.023	0.447	0.53	0.033	0.962			0.005		0.990	0.010
Gellibrand		0.132	0.829	0.039		0.028	0.522	0.45	0.094	0.899	0.007			0.969	0.031
Carlisle		0.275	0.725				0.150	0.850	0.075	0.925				0.900	0.100
Gippsland															
Moondarra			0.900	0.100		0.078	0.575	0.347	0.062	0.938			0.031	0.969	
Holey Plains		0.364	0.636			0.027	0.31	0.664	0.036	0.964				1.000	
Tasmania															
Saltwater			1.000					1.000			1.000			1.000	
Swanport		0.458	0.542			0.042	0.292	0.667		0.417	0.583			1.000	
Cranbrook		0.612	0.388				0.460	0.540		0.896	0.104			1.000	

Table 3 Mean (± SE) and 95% confidence intervals for estimates of nestmate relatedness in the spider *D. ergandros*

Class	$r \pm$ SE	95% CI
All spiders	0.44 ± 0.03	0.39–0.49
Males	0.43 ± 0.03	0.38–0.48
Females	0.37 ± 0.05	0.28–0.47
Adults	0.49 ± 0.05	0.40–0.57
Subadults	0.39 ± 0.04	0.31–0.48
Juveniles	0.49 ± 0.04	0.41–0.56

Alternatively, unrelated spiders may have migrated into the nest.

To examine these possibilities, we tested for differences in the relatedness estimates between the sexes and the age classes. Significant differences may be expected if sex- or age-biased immigration into nests occurred. The results of the tests indicated that the relatedness estimates for the two sexes did not differ significantly ($U = 3867.50$, $P = 0.70$) nor did the estimates for the three age classes ($H_2 = 1.69$, $P = 0.43$), a finding that failed to support the hypothesis that the relatedness estimates below 0.5 resulted from sex- or age-biased migration into nests.

This possibility was explored further by directly examining the genotypes of spiders within nests. Thirteen nests from which only a single individual was sampled were not considered in these analyses. Of the remaining nests, 147 did not contain a putative mother. Of these, 135 contained offspring with genotypes consistent with having been produced by a single mating pair and 12 did not. In all 12 cases, however, a single multiply mated female could have produced the progeny. There were 28 nests with a putative mother, of which 21 contained spiders that could have been produced by the putative mother and a single father, one was consistent with the maternal genotype but required at least two fathers, and six were not consistent with the putative maternal genotype. This latter finding provided direct evidence for acceptance of foreign spiders into *D. ergandros* nests.

Table 4 Hierarchical analysis of genetic structure in the spider *D. ergandros*. The statistics measure the decrease in heterozygosity at different levels of the population due to true inbreeding within locales (F_{IL}), structure of locales within regions (F_{LR}), structure of regions within the total population (F_{RT}), or both inbreeding and population structure (F_{IT})

Locus	F_{IL}	F_{LR}	F_{RT}	F_{IT}
Pgi	0.10	0.11	0.02	0.20
6*Pgd*	0.19	0.23	0.14	0.38
Idh	0.16	0.35	0.06	0.46
Ldh	−0.03	0.01	0.01	−0.01
Overall	0.15	0.23	0.08	0.35

Genetic structure among locales and regions

We first tested the genotypic frequencies within *D. ergandros* locales for deviations from expectations under Hardy–Weinberg equilibrium. A probability test revealed a modest but significant difference from expectations ($P = 0.032$). Examination of the specific alternative hypotheses concerning the nature of the deviation indicated that this departure arose from a deficit ($P < 0.0001$) rather than an excess ($P = 0.99$) of heterozygotes within locales.

Analysis of Wright's hierarchical *F*-statistics further revealed the nature of the genetic structure within and among locales and regions (Table 4). The estimates and associated 95% confidence intervals obtained through our analyses were as follows: $F_{IL} = 0.15$ (0.091, 0.19), $F_{LR} = 0.23$ (0.10, 0.33), $F_{RT} = 0.081$ (0.021, 0.13) and $F_{IT} = 0.35$ (0.18, 0.44). As none of the 95% CIs overlap zero, all the statistics differed significantly from zero. The large coefficient F_{IL} agreed with the above finding of significant heterozygote deficit within locales, and suggested that matings may have occurred among relatives. Estimates of genetic differentiation among locales within regions (F_{LR}) and among regions (F_{RT}) indicated that substantial genetic structure existed at both of these levels of the *D. ergandros* population, and the combined effects of nonrandom mating within locales and genetic structure within and among regions led to a very large overall inbreeding coefficient, F_{IT}. Tests for isolation by distance among locales revealed a significant correlation between genetic distance, as measured by F_{LT}, and geographical distance (Fig. 2, $r_S = 0.24$, $P = 0.006$). This association was not due solely to differentiation between locales from mainland Australia and those from Tasmania, because the association remained significant even when the Tasmanian data were eliminated ($r_S = 0.32$, $P = 0.005$). The relationships among locales were further documented in the dendrogram presented in Fig. 3. The tree supported previous findings in that locales in the same or neighbouring regions tended to be clustered. Perhaps the most notable result arising from the dendrogram

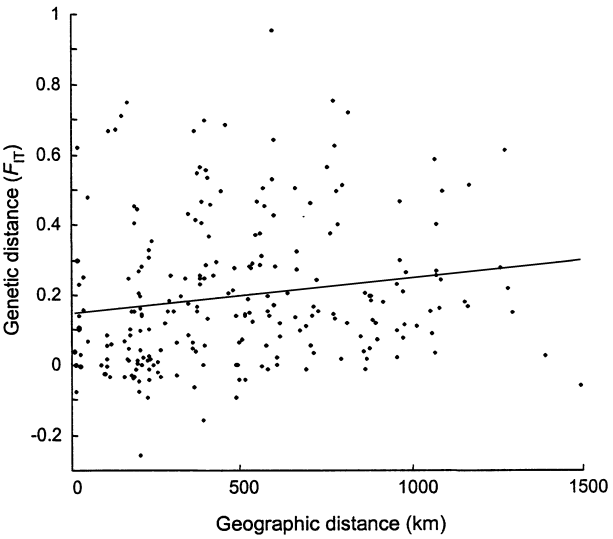

Fig. 2 Relationship between the genetic distance of *D. ergandros* spiders sampled from distinct locales and the geographical distance between locales. The regression line $y = 0.0001x + 0.1485$ has been plotted ($R^2 = 0.0249$).

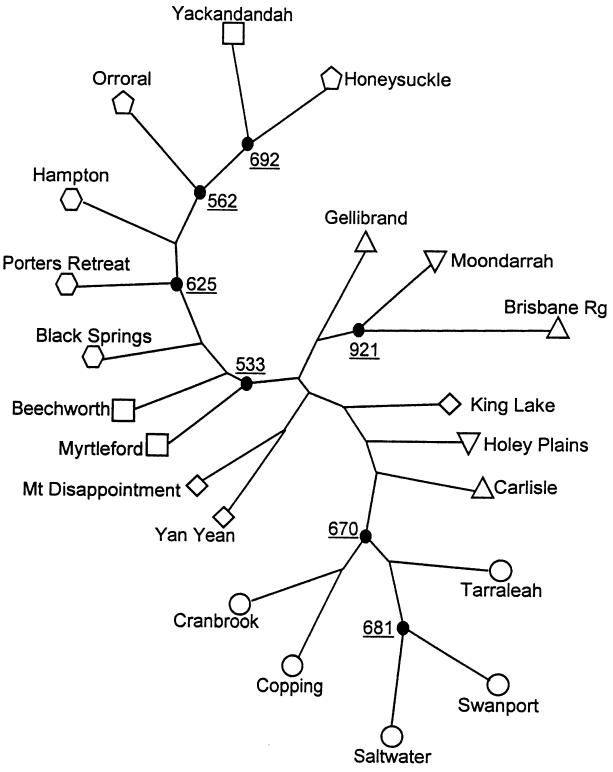

Fig. 3 Unrooted neighbour-joining tree based on genetic distances between *D. ergandros* spiders sampled from distinct locales. Region symbols are as given in Fig. 1; the number of sides of each symbol decreases with increasing latitude, so the geographical proximity of locales is indicated by the number of sides of the symbols (assuming circles have one side). Bootstraps values are placed on nodes (shown as filled black circles) with greater than 50% support.

is the strong support for the node distinguishing all locales from Tasmania from those on the mainland of Australia.

Discussion

Diversity of allozyme markers

Diaea ergandros exhibited relatively low allozymic diversity. The proportions of polymorphic loci (*P*) in other social spiders have been found to vary substantially, with many species possessing particularly few variable loci (range in *P* of 0.045–0.75; Ayre 1977; Lubin & Crozier 1985; Rowell 1985; Smith 1986; Roeloffs & Riechert 1988; Smith & Engel 1994; Smith & Hagen 1996; Johannesen *et al*. 1998). However, the levels of variability observed in social spiders tend to be higher than those in social Hymenoptera (Graur 1985). Estimates of allozyme diversity have also been obtained in several nonsocial spiders (Riechert & Roeloffs 1993; Hudson & Adams 1996; Smith & Hagen 1996; reviewed by Ramirez & Saunders 1999). The variability of markers in these taxa also spans a wide range of values (range in *P* of 0.17–0.67), although the markers tend to be more variable than in social spiders.

Sociality, and the associated inbreeding that accompanies it in some social species, should not lead necessarily to a decrease in the proportion of polymorphic loci within populations, although it may lead to a decrease in observed heterozygosity. Rather, population dynamics involving extinction and foundation of rare, long-lived colonies are suggested as being responsible for the low diversity in some social spiders (Riechert & Roeloffs 1993; Smith & Hagen 1996). However, *D. ergandros* populations do not exhibit these types of dynamics. Rather, *D. ergandros* colonies are short-lived and survive only 1 year (Evans 1995). Thus, perhaps social spiders sometimes display low expected heterozygosities because they have been sampled within geographically circumscribed regions and are poor dispersers, as seen in some nonsocial spiders (Hudson & Adams 1996; Ramirez & Saunders 1999).

Relationships of nestmates

Young *D. ergandros* spiders inhabiting the same nest should be the offspring of one female (Evans 1995). Consequently, the high estimates of nestmate relatedness obtained in this study were broadly consistent with observations of the life history of this spider (Evans 1999). Previous studies documented the relationships of social spiders inhabiting the same nest have, without exception, revealed that nestmates were close relatives. Johannesen *et al*. (1998) and Johannesen & Lubin (1999) found that nestmates were related as approximately half-sibs (0.25) in the subsocial spiders *Eresus cinnaberinus* and *Stegodyphus lineatus*. The relatedness of nestmates in the spider *Agelena consociata* revealed that spiders were related as full sibs (Roeloffs & Riechert 1988; Riechert & Roeloffs 1993) under the observed level of inbreeding. *Anelosimus eximius* possessed extremely high nestmate relatedness values approaching 1.0 in some cases (Smith & Hagen 1996). In addition, Smith & Engel (1994) and Lubin & Crozier (1985) examined the genotypes of *Stegodyphys sarasinorum* and *Achaearanea wau* among nests and found evidence that nestmates were related, although both these studies were hampered by a lack of genetic variation.

Despite the fact that *D. ergandros* nestmates were substantially related, some of the actual relatedness estimates were significantly lower than 0.5, the minimum value expected if all nestmates were full siblings. The most likely explanations for this result are that some females mated multiply or that unrelated individuals migrated into some nests. Closer examination of the genetic data revealed that polyandry could account for the genotypes of nestmates in some cases. Polyandry is fairly common in spiders (Elgar 1998). Moreover, *D. ergandros* females are capable of mating with multiple males. Indeed, in seven of 33 (21%) mating trials, virgin adult females accepted two matings (of a maximum of four males, T. Evans, unpublished).

However, in several cases polyandry could not account for the offspring genotypes observed in *D. ergandros* nests. In 3.5% of the sampled nests, the putative mother heading the nest could not have produced all offspring, and migration of nonrelatives was deemed as the most probable explanation for these cases. The relatedness estimates for the two sexes and for the three life stages (juveniles, subadults, or adults) did not differ significantly, which did not support the hypothesis that sex- or age-biased acceptance of non-nestmate spiders occurred. However, our failure to detect such immigration may result from the relatively low frequency of acceptance of nonrelatives in this study and, consequently, a lack of power. In addition, we note that the estimated frequency of nests containing immigrants represents an underestimate of the actual proportion in the population. As was the case with our ability to detect polyandry, our power to detect acceptance of nonnestmates was fairly low because the putative mother heading each nest was captured only rarely and the genetic markers used were only moderately polymorphic.

The occurrence of unrelated spiders within the nest was somewhat unexpected, as their presence may decrease the inclusive fitness benefits that young spiderlings obtain through the helping behaviour of their mother. Such 'maladaptive' behaviour could occur when, for example, food items caught by the mother to feed her offspring (Evans 1998a,b) are also eaten by nonkin. However, allowing nonkin into nest may provide other benefits to the resident spiders. Indeed, previous studies have demonstrated that spiders inhabiting nests that accept nonkin may enhance their fitness through increased group survival or by using

the foreign spider as a food supply in times of starvation (Evans 1999).

Inbreeding within locales

D. ergandros spiders were substantially inbred within locales, suggesting that relatives mated frequently. This result is particularly intriguing, given the relatively small dimensions of locales (~100 m × 100 m). Non-random mating may be due to the poor migratory abilities of *Diaea* spiders, which must disperse by foot rather than by ballooning (Main 1988; Evans 1995). Over a period of generations, this may lead to a build-up of genetic structure within locales and consequently result in inbreeding within locales. Inbreeding is ubiquitous among other social spiders (Frank 1987; Riechert & Roeloffs 1993; Avilés 1997). In contrast to *D. ergandros*, other social spider taxa often stay and breed within their natal colony (Vollrath 1982; Christenson 1984; Lubin 1986; Roeloffs & Riechert 1988; Avilés 1993; Avilés 1997; Uetz & Hieber 1997), dispersing (usually in groups) only when conditions are favourable (Lubin & Robinson 1982; Vollrath 1982). This strategy leads to consanguineous matings and potentially very high levels of inbreeding (Riechert & Roeloffs 1993; Avilés 1997).

Poor dispersal and inbreeding may lead to local mate competition, whereby related males compete for mating opportunities (Hamilton 1967). Such conditions tend to select for female-biased sex ratios. Indeed, highly female-biased sex ratios are common in other social spiders (Riechert & Roeloffs 1993; Avilés 1997). However, *D. ergandros* males and females are equally frequent (Evans 1998a). Thus the genetic patterns uncovered in this study are not consistent with expectations arising from the observed sex ratios. In addition, the build-up of genetic structure within locales through poor dispersal suggests that spiders inhabiting neighbouring nests will be related relative to spiders inhabiting non-neighbouring nests. However, the presence of such structure would not necessarily be expected to lead to cooperative or helping behaviour among neighbours. Spiders residing in distinct nests probably compete for food, and such competition among relatives may substantially decrease the opportunity for the evolution of helping or cooperative behaviour (Queller 1992; Wilson *et al*. 1992).

Genetic structure among locales and regions

This study documented substantial genetic structure in *D. ergandros* at sampling levels above the nest. Moreover, locales showed a pattern of increasing genetic similarity with proximity. These results are consistent with the poor dispersal ability of *D. ergandros* spiders. Over the long term, low dispersal leads to isolation by distance of local breeding units and strong genetic structure. Higher-level genetic structure has also been observed in several other group-living spiders including *Anelosimus eximius* (Smith & Hagen 1996), *Stegodyphys sarasinorum* (Smith & Engel 1994), *Eresus cinnaberinus* (Johannesen *et al*. 1998) and *Stegodyphus lineatus* (Johannesen & Lubin 1999).

A particularly robust result arising from our higher-level analyses of *D. ergandros* was the strong genetic differentiation between the Tasmanian and the mainland regions. Tasmania was separated from mainland Australia approximately 10 000 years ago (Jennings 1971), and the Bass Straight, which now separates Tasmania from the mainland, would presumably represent a considerable impediment to dispersal at the present time.

Genetic differentiation of taxa located on Tasmania and the mainland has been observed in diverse taxa. Examples of such differentiation have been documented in mammals, such as the quoll *Dasyurus maculatus* (Firestone *et al*. 1999), arthropods, such as the spider *Badumna candida* (Colgan & Gray 1992) and the tick *Ixodes* (Jackson *et al*. 1998), and in both vascular and nonvascular plants, *Atherosperma moschatum* (Shapcott 1994) and *Psilosiphon* (Entwisle *et al*. 2000), respectively. Concordance in genetic structure among many taxa is a strong indication that Tasmania represents a separate biogeographical province from the remainder of mainland Australia (Avise 2000) and therefore deserves more attention in future population genetic studies of the country.

Acknowledgements

We thank Mark Elgar and Michelle Waycott for discussion and comments of previous versions of the manuscript, and Natalie Adamson, Mari Laffer and Nathan Evans for their help collecting spiders. TAE would also like to thank Mike Johnson for the generous use of his laboratory at the University of Western Australia, and Michelle Stuckey for her assistance during this time. This work was supported by the Australian Research Council, Australian Ecology Society, Royal Society of Victoria and the New South Wales Zoological Society (TAE), and a National Science Foundation Postdoctoral Fellowship in the Biosciences no. DBI-9804263 (MADG).

References

d'Andrea M (1987) Social behaviour in spiders. *Monitore Zoologico Italiano Monograph*, **3**, 1–156.

Avilés L (1993) Interdemic selection and the sex-ratio: a social spider perspective. *American Naturalist*, **142**, 320–345.

Avilés L (1997) Causes and consequences of cooperation and permanent-sociality in spiders. In: *The Evolution of Social Behaviour in Insects and Arachnids* (eds Choe JC, Crespi BJ), pp. 476–498. Cambridge University Press, Cambridge.

Avise JC (2000) *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge, MA.

Ayre DJ (1977) *Genetic Heterogeneity Within and Between Populations of* Ixeuticus candidus, 'the common colonial spiders'. Honours Thesis, University of Western Australia, Perth.

Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, NJ.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: Models and estimation procedures. *American Journal of Human Genetics*, **19**, 233–257.

Choe JC, Crespi BJ (1997) *The Evolution of Social Behaviour in Insects and Arachnids*. Cambridge University Press, Cambridge.

Christenson TE (1984) Behaviour of colonial and solitary spiders of the theridiid species *Anelosimus eximius*. *Animal Behaviour*, **32**, 725–734.

Colgan DJ, Gray MR (1992) The genetic structure of the *Badumna candida* group of species (Araneae, Amaurobioidea). *Bulletin of the of the British Arachnological Society of*, **9**, 86–94.

Crozier RH, Pamilo P (1996) *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, Oxford.

Elgar MA (1998) Sexual selection and sperm competition in arachnids. In: *Sperm Competition and Sexual Selection* (eds Birkhead TR, Møller AP), pp. 307–337. Academic Press, New York.

Entwisle TJ, Vis ML, Foard HJ (2000) Biogeography of *Psilosiphon* (Batrachospermales, Rhodophyta) in Australia and New Zealand. *Cryptogamie Algologie*, **21**, 133–148.

Evans TA (1995) Two new social crab spiders (Thomisidae: *Diaea*) from eastern Australia, their natural history and geographic range. *Records of the Western Australian Museum Suppl*, **52**, 151–158.

Evans TA (1997) Distribution of social crab spiders in eucalypt forests. *Australian Journal of Ecology*, **22**, 107–111.

Evans TA (1998a) Factors affecting group survival in social crab spiders. *Biological Journal of the Linnean Society*, **63**, 205–219.

Evans TA (1998b) Offspring recognition by mother crab spiders with extreme maternal care. *Proceedings of the Royal Society of London B*, **265**, 129–134.

Evans TA (1999) Kin recognition in a social spider. *Proceedings of the Royal Society of London B*, **266**, 287–292.

Evans TA (2000) Male work and sex ratio in social crab spiders. *Insectes Sociaux*, **47**, 285–288.

Evans TA, Wallis EJ, Elgar MA (1995) Making a meal of mother. *Nature*, **376**, 299.

Felsenstein J (1989) PHYLIP — phylogeny inference package, version 3.2. *Cladistics*, **5**, 164–166.

Firestone KB, Elphinstone MS, Sherwin WB, Houlden BA (1999) Phylogeographical population structure of tiger quolls *Dasyurus maculatus* (Dasyuridae: Marsupialia), an endangered carnivorous marsupial. *Molecular Ecology*, **8**, 1613–1625.

Frank S (1987) Demography and sex ratio in social spiders. *Evolution*, **41**, 1267–1281.

Graur D (1985) Gene diversity in the Hymenoptera. *Evolution*, **39**, 190–199.

Hamilton WD (1964) The genetical evolution of social behaviour I–II. *Journal of Theoretical Biology*, **7**, 1–52.

Hamilton WD (1967) Extraordinary sex ratios. *Science*, **156**, 477–488.

Hudson P, Adams M (1996) Allozyme characteristics of the salt lake spiders (*Lycosa*: Lycosidae: Araneae) of southern Australia: systematics and population genetic implications. *Australian Journal of Zoology*, **44**, 535–567.

Jackson J, Chilton NB, Beveridge I, Morris M, Andrews RH (1998) An electrophoretic comparison of the Australian paralysis tick, *Ixodes holocyclus* Neumann, 1899, with *I. cornuatus* Roberts, 1960 (Acari: Ixodidae). *Australian Journal of Zoology*, **46**, 109–117.

Jennings JN (1971) Sea level changes and land links. In: *Aboriginal Man and the Environment* (eds Mulvaney DJ, Golson J), pp. 1–13. Australian National University Press, Canberra.

Johannesen J, Baumann T, Seitz A, Veith M (1998) The significance of relatedness and gene flow on population genetic structure in the subsocial spider *Eresus cinnaberinus* (Araneae: Eresidae). *Biological Journal of Linnean Society*, **63**, 81–98.

Johannesen J, Lubin Y (1999) Group founding and breeding structure in the subsocial spider *Stegodyphus lineatus* (Eresidae). *Heredity*, **82**, 677–686.

Kullman EJ (1968) Soziale phaenomene bei spinnen. *Insectes Sociaux*, **15**, 289–297.

Kullman EJ (1972) Evolution of social behavior in spiders (Araneae; Eresidae and Theridiidae). *American Zoologist*, **12**, 419–426.

Lewis PO, Zaykin D (2000) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data*, Version 1.0 (d15).

Lubin YD (1986) Courtship and alternative mating tactics in a social spider. *Journal of Arachnology*, **14**, 239–257.

Lubin YD, Crozier RH (1985) Electrophoretic evidence for population differentiation in a social spider *Achaearanea wau* (Theridiidae). *Insectes Sociaux*, **32**, 297–304.

Lubin YD, Robinson MH (1982) Dispersal by swarming in a social spider. *Science*, **216**, 319–321.

Main BY (1988) The biology of a social thomisid spider. In: *Australian Arachnology* (eds Austin AD, Heather NW), pp. 55–73. Australian Entomological Society Miscellaneous Publications no. 5. Australian Entomological Society, Brisbane.

Queller DC (1992) Does population viscosity promote kin selection? *Trends in Ecology and Evolution*, **7**, 322–324.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Ramirez MG, Saunders TA (1999) Allozyme diversity in non-social spiders: pattern, process and conservation implications. *Journal of Insect Conservation*, **3**, 327–340.

Raymond M, Rousset F (1995) GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Richardson BJ, Baverstock PR, Adams M (1986) *Allozyme Electrophoresis: a Handbook for Animal Systematics and Population Studies*. Academic Press, Sydney.

Riechert SE, Roeloffs RM (1993) Evidence for, and consequences of, inbreeding in the cooperative spiders. In: *The Natural History of Inbreeding and Outbreeding* (ed. Thornhill N), pp. 283–303. University of Chicago Press, Chicago.

Roeloffs R, Riechert SE (1988) Dispersal and population–genetic structure of the cooperative spider *Agelena consociata* in west African rainforest. *Evolution*, **42**, 173–183.

Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. *Genetics*, **140**, 1413–1419.

Rowell DR (1985) *Chromosomal Structure Sociality in Delena Cancerides* (Araneae: Sparassidae). PhD Thesis, Australian National University, Canberra.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Shapcott A (1994) Genetic and ecological variation in *Atherosperma moschatum* and the implications for conservation of its biodiversity. *Australian Journal of Botany*, **42**, 663–686.

Shear WA (1970) The evolution of social phenomena in spiders. *Bulletin of the of the British Arachnological Society of*, **1**, 65–76.

Smith DR (1986) Population genetics of *Anelosimus eximius* (Araneae, Therididdae). *Journal of Arachnology*, **14**, 201–218.

Smith DR, Engel MS (1994) Population structure in an Indian cooperative spider *Stegodyphus sarasinorum* Karsch (Eresidae). *Journal of Arachnology*, **22**, 108–113.

Smith DR, Hagen RH (1996) Population structure and interdemic selection in the cooperative spider *Anelosimus eximius*. *Journal of Evolutionary Biology*, **9**, 589–608.

Uetz GW, Hieber CS (1997) Colonial web-building spiders: balancing the costs and benefits of group-living. In: *The Evolution of Social Behaviour in Insects and Arachnids* (eds Choe JC, Crespi BJ), pp. 458–475. Cambridge University Press, Cambridge.

Vollrath F (1982) Colony foundation in a social spider. *Zeitschrift für Tierpsychologie*, **60**, 313–324.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer Associates, Sunderland.

Wilson DS, Pollock GB, Dugatkin LA (1992) Can altruism evolve in purely viscous populations? *Evolutionary Ecology*, **6**, 331–341.

This work is part of a broader study on *Diaea* social spiders originating from the dissertation research of Theodore Evans. Both authors continue to be interested in the social biology of arthropods. Michael Goodisman is currently a postdoctoral fellow at the University of Arizona investigating social insect evolution and development. Theodore Evans is now an Australian Research Council Postdoctoral Fellow at the Commonwealth Scientific and Industrial Research Organization of Australia studying termite organization and behaviour.
